cell having been synthesized *in situ* and being attached to the surface of the support through a covalent linkage, where the sequence of the oligonucleotides of a first cell is different than the sequence of the oligonucleotides of a second cell,

which method comprises applying the polynucleotide under hybridization conditions to the support and observing the location of hybridized polynucleotide on the support.

62. A method of analyzing a polynucleotide, by the use of apparatus comprising a support segregated into at least two defined cells, each cell having attached thereto oligonucleotides containing predetermined sequences, the oligonucleotides of each cell having a length of 8 to 20 nucleotides, where the sequence of the oligonucleotides of a first cell is different than the sequence of the oligonucleotides of a second cell,

which method comprises applying the polynucleotide under hybridization conditions to the support and observing the location of hybridized polynucleotide on the support.--

REMARKS

Favorable reconsideration is respectfully requested.

Upon entry of this amendment, the claims will be 41 to 52 and 54 to 62.

Applicant acknowledges with appreciation the helpful interview with Examiner Marschel on July 12, 1996, which was

attended by the inventor, Dr. E.M. Southern, his British patent agent, P. Pennant, and undersigned.

This Supplemental Response addresses all substantial issues raised during the course of the interview.

In the above amendment, the following changes are proposed:

Claims 40 and 53 have been cancelled without prejudice to the subject matter thereof.

New claims 60, 61 and 62 are method claims, corresponding to apparatus claims 41, 42 and 43. No new issue is raised by addition of these method claims.

Claims 41, 42 and 43 are amended to replace the phrase "defined sequence" with alternative language discussed at the interview.

In the first part of the interview, the issues raised in the Official Action of December 28, 1995 and replied to in the response dated May 28, 1996 were discussed.

In this regard:

1. The Examiner indicated that the new title submitted by applicant was acceptable.

2. The Examiner stated that claims 44 to 48 were not supported by the specification. In reply, Dr. Southern pointed to two passages in the British priority application, namely page 7, line 34 to page 8, line 13 and page 11, lines 9 to 29. He pointed out that these two passages together provided support

for all the features recited in claims 44 to 48. If further support were needed, it was provided in the international application PCT/GB89/00460 (WO 89/10977) in Example 3 at page 17, line 25 to page 19, line 16 and in Table 1 at page 25. The Examiner indicated that these arguments were indicative of support for the claims in issue.

With regard to the rejection of the phrase "defined sequence" as indefinite, the Examiner contended that any known polynucleotide is inherently capable of being sequenced and therefore inherently has a defined sequence. Applicant proposed alternative claim language where "with defined sequence" or "with known sequence" was replaced by "containing predetermined sequences". The Examiner indicated that this change would meet the rejection. This alternative language now appears in claims 41 to 43 and 60 to 62.

In the Official Action, the Examiner had identified various priority dates for different claims, and had stated, in particular, that new claims 44 to 48 were not entitled to any date earlier than May 3, 1991. At the interview, the Examiner indicated that claims 44 to 48 were entitled to a date of May 2, 1989 (or perhaps of May 3, 1988). Applicant replied that all claims were fairly based on their British priority application 8810400.5, filed on May 3, 1988, and that in any event, the priority date issue was not important in relation to any outstanding objection/rejection.

The Examiner pointed out that claim 41 recites that the oligonucleotides of each cell are attached to the surface of the support through a terminal nucleotide, but without saying that the attachment is by a covalent linkage. He questioned whether any attachment other than a covalent linkage was possible and further questioned whether the claim was enabled. Dr. Southern replied that attachment of oligonucleotides to solid supports by a biotin-streptavidin link, or by an immune antigen-antibody link, were perfectly possible and were indeed used. In the light of this argument, the Examiner indicated that this was responsive to his inquiry.

The prior art cited in the previous Official Action was next discussed.

Mundy discloses a membrane section with four spots on each section, two spots with pBR 322 DNA attached and two spots with pAT 153 DNA attached. The Examiner contended that this disclosure anticipated claim 40. But applicant has now deleted claim 40. The Examiner also cited Mundy as relevant against claims 41, 42 and 43. Applicant replied that each of these claims contains features unobvious from Mundy as follows:

Claim 41 recites that the oligonucleotides of each cell are attached to the surface of the support through a terminal nucleotide;

Claim 42 recites that the oligonucleotides of each cell have been synthesized *in situ* and are attached to the surface of the support through a covalent linkage;

Claim 43 recites that the oligonucleotides of each cell have a length of 8 to 20 nucleotides.

In the light of these distinctions, the Examiner indicated that claims 41 to 43 were unobvious from Mundy. The same is considered to be the case for the corresponding method claims 60 to 62.

Saiki shows arrays of immobilized PCR amplification products 110 nucleotides in length. Applicant's claims 41 to 43 distinguish over Saiki in the same way as over Mundy. The Examiner indicated that applicant's claims 41, 42 and 43 distinguished over Saiki.

The Examiner did not seek to apply the Brigati reference against any claim.

The Examiner cited Hafeman, taken together with Macevicz and Wood as rendering obvious claims 40, 43 (plus 49 and 50) and 53 (plus 57 and 59). The basis for this rejection is that Macevicz describes a standard dot-blot assay format, in which a target is immobilized and addressed by labeled probes, typically 7 to 11 bases in length, in solution. In the light of Hafeman, it would be obvious to immobilize the probes (rather than the target) and address them by a labeled target (rather than labeled probes) in solution. In reply, Dr. Southern pointed

out that this role-reversal was not taught or suggested by Hafeman and was not obvious. Specifically, it would not be obvious to make an array of oligonucleotides for the purpose described by Macevicz from a reading of Hafeman. Macevicz requires large numbers of short oligonucleotides to take part in a hybridization reaction. Hafeman does not teach a manner of attachment, particularly one that would allow for the large numbers required; nor does he teach the manner of attachment, e.g., through a terminal nucleotide, that is necessary to allow such short oligonucleotides to take part in a hybridization reaction. A person skilled in the art, reading these two references, would have to provide solutions to both of these problems (and others) to realize the present invention. Wood clearly does not overcome these deficiencies. This argument was indicated to overcome the above rejection.

With regard to Fodor, it is only relevant to present claims 44 to 48. Since claims 44 to 48 have a priority date of no later than May 2, 1989, it was indicated by the Examiner that Fodor was not citable against them.

In the Official Action, the Examiner had objected to various informalities in the disclosure. Applicant understands that these objections have been met by their response of May 28, 1996.

Next, the Examiner turned to the Information Disclosure Statement of May 28, 1996 and stated that he had reviewed all the

references contained therein, and asked for further comments on three of them, as will now be discussed.

Dattagupta (EP 235 726) discloses a reverse dot-blot assay technique, in which probes are immobilized on a support and addressed by a labeled polynucleotide to be analyzed in solution. The probes are nucleic acids or oligonucleotides, and are discussed at page 9, lines 6 to 40. The main teaching concerns probes which are nucleic acids of substantial length. A paragraph at page 9, lines 34 to 41 refers to oligonucleotides as probes and to the difficulty of immobilizing oligonucleotides on solid supports. There is no general discussion of the lengths of the oligonucleotides.

In the experimental section, most Examples including Examples 5, 8, 11, 13 are concerned with the use of nucleic acids of indeterminate length as probes. Only in Examples 6 and 7 are oligonucleotides used as probes. Applicant has commented on these two examples - see the text, "Applicant's Commentary", pages 10 to 15, filed with applicant's response of May 28, 1996. In these examples, 43-mers or longer oligonucleotides were used as probes. Applicant surmises that Dattagupta needed to use oligonucleotide of such length in order to get them properly immobilized on a solid surface in a form in which they could take part in hybridization reactions. There is no teaching or suggestion to use shorter oligonucleotides.

The Examiner indicated that Dattagupta might be relevant to claim 40, but applicant has now deleted this claim. The remaining claims contain one or more features neither disclosed nor suggested by Dattagupta, e.g., the use of oligonucleotides of 8 to 20 nucleotides in length.

Huang (U.S. 4,328,073) discloses a method and device for simultaneously assaying biological fluids for a variety of substances using a support on which compounds reactive for the various substances are immobilized in preselected areas. There is a reference to complementary DNA at column 2, line 41, and references to nucleosides, nucleotides, DNA and RNA in Table 3 bridging pages 5 and 6. But no detailed information is given, and it is quite unclear how assays for or using these substances might be performed. More importantly, the reference is specifically directed to the simultaneous quantitative analysis of a plurality of substances. In contrast, the present application is concerned with investigating the sequence of a single polynucleotide. These two approaches are incompatible, and one does not suggest the other.

Gingeras (WO 88/1302) discloses novel supports for end-attachment of oligonucleotide probes for use in sandwich hybridization assays for detecting nucleic acids. The supports disclosed are all particulate (beads, see the Abstract). There is no teaching or suggestion to provide a support segregated into defined cells, each cell having attached thereto oligonucleotides

contain in predetermined sequences with different cells containing different oligonucleotides. There is no teaching or suggestion to investigate the sequence of a target nucleic acid.

No further issues remaining, allowance of this application is respectfully requested.

If the Examiner has any comments or proposals for expediting prosecution, he is invited to contact the undersigned at the telephone or facsimile number below.

Respectfully submitted,

Edwin SOUTHERN

By _____
Matthew Jacob
Registration No. 25,154
Attorney for Applicant

MJ/act
Washington, D.C.
Telephone (202) 371-8850
Facsimile (202) 371-8856
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